Uptake and Fate of Ascorbic Acid-2-Phosphate in Infiltrated Fruit and Vegetable Tissue

G.M. SAPERS, R.L. MILLER, F.W. DOUGLAS, JR, and K.B. HICKS

ABSTRACT

Ascorbic acid-2-phosphate (AAP) and ascorbic acid (AA) were infiltrated into apple and potato tissue to control browning. Apple tissue absorbed more AAP and AA than potato under similar conditions. AAP hydrolysis by endogenous acid phosphatase (APase) yielded AA which accumulated or became oxidized to dehydroascorbic acid, depending on the rate of hydrolysis and browning tendencies of samples. APase activity varied greatly with commodity, method of sample preparation and sample pH. Variation in the ability of AAP to inhibit browning in different products could be explained by these factors.

INTRODUCTION

THE ASCORBIC ACID-2-phosphates are highly effective inhibitors of enzymatic browning at cut surfaces of raw apple fruit (Sapers et al., 1989a, 1989b); however, ascorbic acid-2-phosphate (AAP) and ascorbic acid-2-triphosphate (AATP) are less effective than ascorbic acid (AA) as browning inhibitors for cut potato (Sapers, 1987). These esters are considered stable sources of AA, releasing ascorbic acid only when hydrolyzed by acid phosphatase (APase) (Seib and Liao, 1987). APase activity in fruit and vegetable tissue is a function of the enzyme concentration, substrate specificity, cellular location, pH (Sugawara et al., 1981; Crasnier and Giordani, 1985; Paul and Williamson, 1987), and concentrations of multivalent cations (Tu et al., 1988). Knowledge of the extent to which AAP is absorbed by treated fruits and vegetables and of their endogenous APase activity would facilitate optimization of AAP treatments for other commodities besides apple. Our objective was to elucidate the fate of AAP in infiltrated apple and potato plugs, and to investigate endogenous APase as a factor limiting applicability of AAP treatments to control enzymatic browning.

MATERIALS & METHODS

Raw materials

Apple, pear and mushroom samples, representing common cultivars, were obtained locally and refrigerated briefly. Potatoes designated as Russet, Red Bliss or "all purpose" also were obtained locally; Russet Burbank tubers were obtained from the Aroostook Experimental Farm, Presque Isle, ME.

Dipping experiments

Immediately prior to treatment, plugs were cut from 4–6 apples or potatoes with an electric cork borer, using a 22 mm ID stainless steel cutting tube (Sapers and Douglas, 1987). Plugs were trimmed to remove peel, skin, or core portions and submerged in 500 mL of 56.8 mM AA, dehydroascorbic acid (DHAA) or AAP solution for 90 sec. After dipping, the plugs were drained in a plastic colander for ca 30 sec, and the colander screen blotted carefully with paper toweling to remove adhering solution. Failure to remove this residual solution from the screen resulted in highly variable uptake. Treated plugs were placed in a dry colander, covered with aluminum foil to minimize dehydration, and stored at room temperature (ca 20°C) up to 48 hr. Samples of 4 to 6 plugs were removed for analysis immediately after treatment and after 2 1/2, 5, 24 and 48 hr of storage. Additional analyses were performed on untreated plugs, taken from the same lot of apples or potatoes as the treated plugs. Duplicate dipping trials were carried out with plugs from Granny Smith apples (slow browning), Red Delicious apples (rapid browning), and Russet Burbank potatoes (rapid browning).

Authors Sapers, Miller, Douglas (deceased) and Hicks are with the U.S. Department of Agriculture, ARS, Eastern Regional Research Center, 600 E. Mermaid Lane, Philadelphia, PA 19118,

Preparation of juice containing AAP

Raw juice was prepared from Granny Smith apples with an Acme Supreme Juicerator (Acme Juicer Mfg. Co., Lemoyne, PA), lined with a Whatman No. 1 filter paper strip (Sapers and Douglas, 1987). To prevent browning during application of treatments to samples, 0.28 mM AA was added during juicing. AAP (1.14 mM) was added to the juice which was then held at 20°C for 6 hr. Samples of juice were analyzed for AA and AAP initially and after 2, 4 and 6 hr. Browning in the juice was measure by tristimulus colorimetry with a Gardner XL-23 colorimeter, as described by Sapers and Douglas (1987).

Determination of AA, AAP and DHAA in plugs and juice

Samples of treated or control apple or potato plugs weighing about 30g were blended with 60 mL of HPLC mobile phase (acetonitrile - 0.05M KH_2PO_4 (75:25)) and 30 mL 2.5% metaphosphoric acid to extract AA, DHAA and AAP. Extracts were prepared for analysis by filtration and passage through a C_{18} Sep-Pak cartridge, as described previously (Sapers et al., 1990). Juice samples weighing about 30g were prepared for analysis by the same procedure but with omission of blending.

AA and AAP were determined in duplicate in plug extracts or juice by HPLC on an aminopropyl bonded-phase silica column eluted isocratically with mobile phase (Sapers et al., 1990). DHAA was determined by difference following reduction to AA with dithiothreitol (DTT) and measurement of total AA. Concentrations of AA and AAP were calculated from sample peak areas with appropriate dilution factors and calibration factors determined by repeated injections each day of an external standard containing 0.28 mM AA and 0.28 mM AAP in acetonitrile—1.12% metaphosphoric acid solution (1:1). Endogenous AA and DHAA in apple and potato tissue were determined by analysis of untreated control plugs. Differences in AA and AAP uptake among apple and potato samples were examined by the Bonferroni LSD mean separation test (Miller, 1981), applied both to columns (cultivar effects) and rows (AA vs AAP).

Acid phosphatase assay

Assays for APase were performed on samples of apple, pear, and potato homogenates and on their juice by a colorimetric method (Sigma Procedure No. 104, Sigma Chemical Co., St. Louis). In this procedure, the substrate, p-nitrophenol phosphate, was hydrolyzed to p-nitrophenol in pH 4.8 citrate buffer at 37°C. After 30 min, the solution was made alkaline to stop the reaction and develop the color, which was measured at 410 nm. Juices were prepared with the juicerator from sliced fruits or tubers that had been dipped in 0.1M AA solution to prevent browning, which would interfere with the absorbance measurement. About 50 mL of juice was collected in tared beakers containing 150 mL 0.1M AA, used as diluent. Dilution factors were determined from the weight of collected juice. Further dilutions were made with 0.1M AA, as required. To determine the effect of pH on APase activity in apple, assays were performed on Granny Smith and Red Delicious juices, with substitution of 90 mM citrate buffer, adjusted to pH between 3 and 4 with 10% NaOH, for the pH 4.8 90 mM citrate buffer normally used.

Table 1—Uptake of ascorbic acid and ascorbic acid-2-phosphate in infiltrated apple and potato plugs[a]

	Uptake (μmoles/100 g)	
	Ascorbic acid[b]	Ascorbic acid-2-phosphate
Granny Smith apple	308[cD]	360[dC]
Red Delicious apple	309[cD]	457[cC]
Russet Burbank potato	168[dC]	166[eC]

[a] Dipped in 56.8 mM ascorbic acid or ascorbic acid-2-phosphate for 90 sec.
[b] Corrected for endogenous ascorbic acid.
[c-e] Means of duplicate determinations for duplicate trials; means within columns, followed by different lower case superscripts, or means within rows, followed by different capitalized superscripts, are significantly different at $p < 0.05$ by the Bonferroni LSD test.

To compare juice and homogenates prepared from the same apple, pear or potato samples, a 20g portion of each subdivided sample, previously dipped in 0.1M AA, was blended with 60 ml 0.1M AA for 2 min at low speed with a Waring Blendor. Homogenates were diluted with 0.1M AA as required and assayed for APase using an 0.4 ml aliquot of sample and 2× the specified reagent volumes. To avoid interference from suspended solids during absorbance measurements, the assay mixtures were clarified after incubation and NaOH addition by addition of 0.24g Celite Analytical Filter Aid (Fisher) followed by vacuum filtration through Whatman No. 50 paper. APase in mushrooms was assayed by a similar procedure, but homogenates were prepared at 4°C and were more highly diluted because of greater APase activity. The assay mixture was clarified by centrifugation for 1 min at 3200 rpm prior to measurement of absorbance.

Addition of AAP and APase to Granny Smith juice

The extent of enzymatic browning in 30 ml portions of raw Granny Smith apple juice was determined by tristimulus colorimetry (Sapers et al., 1989a). Percent inhibition values, indicative of browning inhibitor effectiveness, were calculated from measurements of the a-value, which increases in browning apple juice. In Experiment I, samples containing 1.14 mM AA or 1.14 mM AAP were compared with an untreated control. In Experiment II, samples containing 1.14 mM AAP or 1.14 mM AAP + 8 units of sweet potato APase (Sigma), dissolved in 1 ml 1% bovine serum albumin, were compared with a control.

RESULTS & DISCUSSION

Uptake and fate of AA and AAP in infiltrated plugs

The uptake of AA and AAP by apple and potato plugs was determined by HPLC analysis of the treated plugs (Table 1). Endogenous AA levels in untreated apple plugs were 6–8 μmoles/100g; Russet Burbank plugs contained 52 μmoles endogenous AA/100g. Apple plugs absorbed significantly more AA or AAP than did potato plugs. This may have been a consequence of greater internal air space in apple, as evidenced by its lower specific gravity, ca. 0.8 (Dewey et al., 1966), compared to 1.08 for Russet Burbank potato (Smith, 1987). Wiley and Binkley (1989) reported that apple contained ca. 25% occluded gas by volume. AAP uptake was greater than AA uptake in apple but not in potato plugs. This difference may be related to the structure and/or charge of the browning inhibitor molecule. Uptake of these compounds was relatively uniform trial to trial; in 3 successive dipping trials, Russet Burbank plugs absorbed 147, 190 and 199 μmoles AA per 100g (corrected for endogenous AA).

During storage of plugs infiltrated with AA, the AA concentration gradually became depleted (Fig. 1) as a consequence of oxidation by quinones generated from polyphenols by polyphenol oxidase (Ponting and Joslyn, 1948) and other oxidation reactions. Absorbed AAP was hydrolyzed to AA, presumably by endogenous acid phosphatase, resulting in a gradual build-up of AA to levels which, in apple, exceeded those seen in AA-infiltrated plugs at corresponding storage times. This build-up was followed by a decrease, probably due to AA oxidation, between 24 and 48 hr. In Granny Smith plugs (Fig. 1A), which brown very slowly, the rate of depletion of infiltrated AA was less and the build-up of AA from AAP hydrolysis was greater than in Red Delicious plugs (Fig. 1B) which brown rapidly. Russet Burbank potato plugs infiltrated with AA (Fig. 1C) showed a gradual loss of AA; plugs infiltrated with AAP showed a delayed build-up of AA above the level that represents endogenous AA (ca. 50 μmoles/100g). These results were consistent with the rapid browning seen in Russet Burbank plugs.

With both apple and potato, plugs infiltrated with AA tended to increase in AA content between 5 and 48 hr following the initial decrease, a possible indication of ascorbate free-radical reductase activity (Borraccino et al., 1986). To examine this further, Granny Smith apple plugs were dipped in 56.8 mM

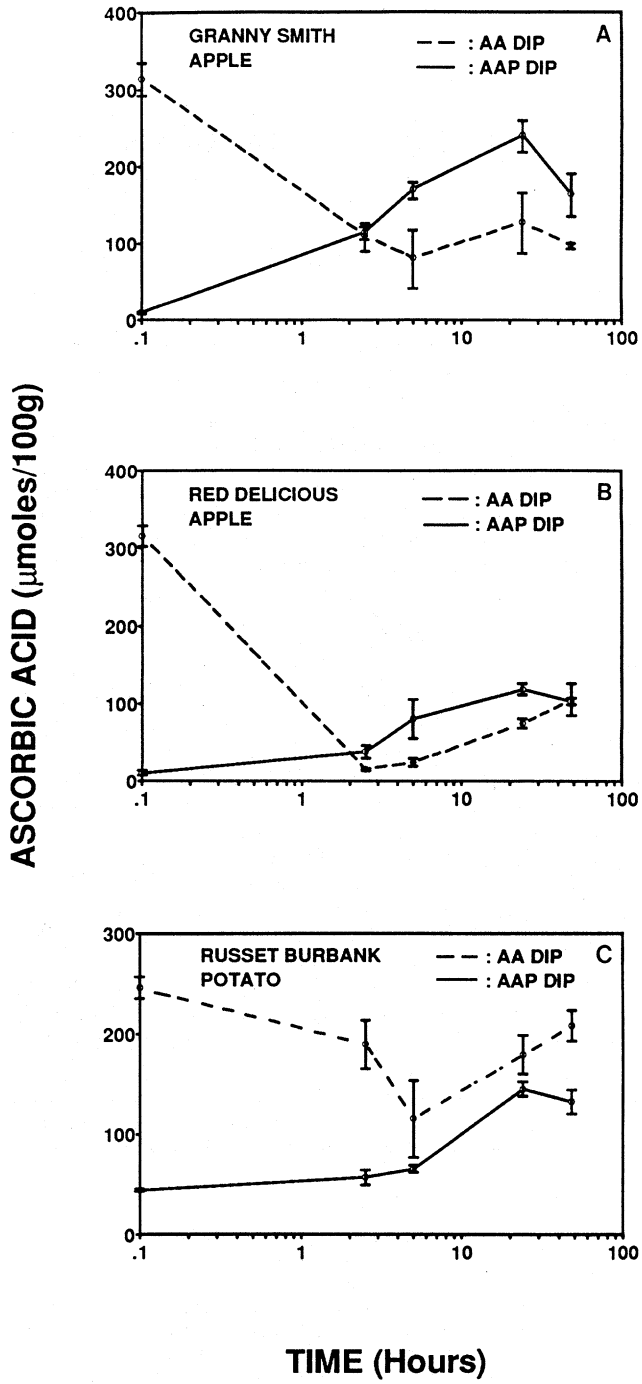

Fig. 1—Ascorbic acid concentrations in apple and potato plugs, dipped in 56.8 mM ascorbic acid (---) or ascorbic acid-2-phosphate (—) and stored at 20°C. Concentration values include endogenous ascorbic acid.

Table 2—Dehydroascorbic acid (DHAA) in apple and potato plugs infiltrated with ascorbic acid (AA) or ascorbic acid-2-phosphate (AAP)

		AA dip		AAP dip	
	Storage (hr)	DHAA (μmoles/100 g)[a]	Percent of added AA	DHAA (μmoles/100 g)[a]	Percent of hydrolyzed AAP[b]
Granny Smith apple	0	0	0	1	–
	2.5	200	65	20	31
	5	175	57	26	15
	24	118	38	5	1
	48	46	15	16	4
Red Delicious apple	0	6	2	0	–
	2.5	267	86	28	47
	5	243	79	22	14
	24	153	50	54	14
	48	49	16	16	4
Russet Burbank potato	0	0	0	1	–
	2.5	23	14	31	42
	5	104	62	48	40
	24	2	1	0	0
	48	10	6	8	5

[a] Corrected for endogenous DHAA.
[b] Based on AAP concentrations before and after storage.

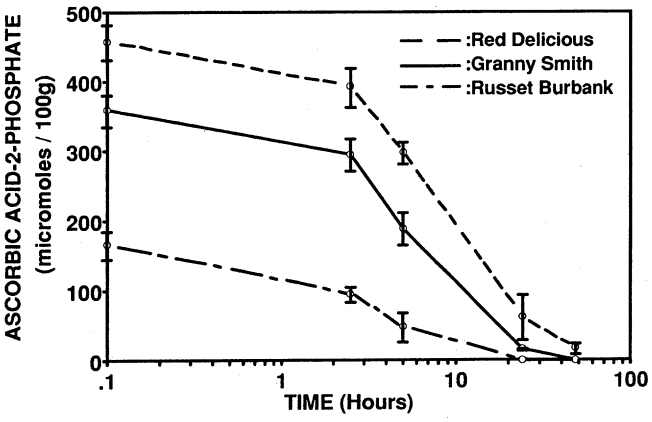

Fig. 2—Ascorbic acid-2-phosphate (AAP) concentrations in apple and potato plugs, dipped in 56.8 mM AAP and stored at 20°C.

Table 3—Acid phosphatase activity in selected fruits and vegetables

			Activity (U/100 g)[a]	
Commodity	Cultivar	n	Mean	Range
Apple	Golden Delicious	12	11	7-16
	Granny Smith	17	7	3-17
	Idared	12	9	5-16
	McIntosh	12	12	5-21
	Red Delicious	12	9	3-20
	Rome	12	12	10-16
	Winesap	11	14	11-18
Pear	Anjou	10	73	46-120
	Bartlett	12	95	58-139
	Bosc	12	59	33-89
Potato	"All purpose"	12	395	258-550
	Red Bliss	11	294	245-354
	Russet	11	196	158-296
Mushroom[b]	Cultivated	9	803	745-862

[a] One unit (U) APase required to release 1 μmole paranitrophenol/min under conditions of assay.
[b] Assay performed on homogenates; assays performed on juice for all other commodities.

DHAA for 90 sec, resulting in a DHAA uptake of 194 μmole/100g, and stored at 20°C for 24 hr. No AA could be detected in the plugs initially, but after 2.5, 5 and 24 hr, they contained 12, 14 and 42 μmoles AA/100g, respectively. Clearly a mechanism for reduction of DHAA to AA in apple tissue, held under conditions favoring AA oxidation at the cut surface, was present.

DHAA concentrations in infiltrated plugs increased rapidly during storage, accounting for a large percentage of the absorbed AA or AA generated by AAP hydrolysis (Table 2). The higher concentrations of DHAA found in Red Delicious plugs, compared to Granny Smith plugs, was consistent with the greater extent of browning in Red Delicious. After 5 hr storage, DHAA concentrations in plugs infiltrated with AA decreased greatly, due both to reduction to AA (Fig. 1) and to further oxidation (difference between DHAA loss and AA increase). Plugs infiltrated with AAP generally contained lower concentrations of DHAA than did plugs infiltrated with AA. Previously, we have shown that AAP was more effective than AA as a browning inhibitor for cut apple (Sapers et al., 1989a). The fact that DHAA accumulation was proportionately less in apple tissue infiltrated with AAP, compared to AA, was consistent with the gradual release of AA from AAP in treated samples.

Initial rates of AAP hydrolysis in infiltrated apple and potato plugs appeared similar (Fig. 2). After 5 hr storage, however, AAP concentrations remaining in infiltrated Granny Smith plugs were 51%, and in Red Delicious, 66% of the original concentrations, as compared to 29% in Russet Burbank. About 40% of the hydrolyzed AAP in potato was oxidized to DHAA during the first 5 hr, compared to about 15% for apple plugs. Apparently, AA generated at potato cut surfaces from absorbed AAP was oxidized very rapidly by quinones, AA oxidase or autoxidation reactions. These results and the relatively small AAP uptake in potato may explain why AAP was relatively ineffective as a browning inhibitor for potato.

Acid phosphatase in fruits and vegetables

Since the effectiveness of AAP as a browning inhibitor depends on hydrolysis to AA by endogenous APase, assays for this enzyme were performed on representative fruits and vegetables that are subject to enzymatic browning (Table 3). Of the commodities surveyed, apple contained the least APase, and mushroom had the highest activity. Variation in activity within varieties was at least as great as variation among varieties. Günther and Burckhart (1968) reported similar results for juice from 11 apple varieties and found that APase activity increased from the peel toward the core. The relatively high APase activity found in potato tubers was consistent with the rapid disappearance of AAP in infiltrated plugs within 24 hr but not with the relatively similar hydrolysis rates seen in apple and potato plugs during the first 5 hr (Fig. 2). Further study is needed to explain this discrepancy.

With exception of mushrooms, these assays were performed on raw juices prepared from each sample. AAP was not an effective browning inhibitor in apple juice, in contrast to its behavior on the cut surface of apple fruit (Sapers et al., 1989a). To determine whether this difference was related to the APase activity, we assayed juices and homogenates prepared from

Table 4—Acid phosphatase activity in juice and homogenate preparations of apple, pear and potato samples

		Activity (U/100g)[a]		
	Sample	Homogenate	Juice	Homogenate/Juice
Granny Smith apple	1	42	14	3.0
	2	18	4	4.1
Red Delicious apple	1	32	10	3.2
	2	64	19	3.4
Bartlett pear	1	40	32	1.2
	2	65	39	1.7
Russet potato	1	82	220	0.4
	2	116	177	0.7

[a] Means of duplicate assays.

Table 5—Effects of ascorbic acid-2-phosphate (AAP), ascorbic acid (AA), and acid phosphatase (APase) on enzymatic browning in Granny Smith apple juice stored at 20°C

		Percent inhibition (a-value)		
		Storage (hr)		
Expt.	Treatment	1	2	4
I	1.14 mM AA	95	95	82
	1.14 mM AAP	82	63	59
II	1.14 mM AAP	89	71	68
	1.14 mM AAP + APase[a]	94	93	93

[a] 1 mL 1%. bovine serum albumin solution containing 8 units APase added to 30 mL juice.

single composite samples of apples and other commodities (Table 4). With Granny Smith and Red Delicious apples, homogenates contained 3-4 times the APase activity in juice. Differences in APase activity between juice and homogenates of pears and potatoes were not so great. The apple data suggested that a major proportion of the APase activity was bound to cell walls or membranes that were removed by filtration during juice preparation. Hence, AAP hydrolysis would be insufficient to replace AA lost through oxidation reactions.

The occurrence of bound APase has been reported in various plant tissues including yam tuber membranes (Kamenan and Diopoh, 1983) and cell walls from cultured sycamore cells (Crasnier and Giordani, 1985), potato tubers (Sugawara et al., 1981), and corn (Tu et al., 1988). Hislop et al. (1979) reported that acid phosphatase in cultured apple cells, visualized for electron microscopy by a lead-capture method, was located in intercellular spaces or in cell walls where it was either uniformly distributed or associated with the middle lamellae and the plasmalemma/wall interface.

The true APase activity in apple juice may be substantially less than that indicated by the assay procedure because of the low pH of juice (Lopez et al., 1958; Mattick and Moyer, 1983). The APase activity of Granny Smith juice, determined at the juice pH of 3.5 was 4.2 U/100g, about 40% of the activity determined at the assay pH of 4.8, 10.3 U/100g. With Red Delicious, the APase activity of juice at pH 4.1 was 7.3 U/100g, about 60% of the activity at the assay pH (11.8 U/100g). These results were similar to pH effects on APase activity reported by Günther and Burckhart (1968).

Evidence in support of the hypothesis that insufficient APase activity limits the effectiveness of AAP in apple juice was obtained by adding sweet potato APase to Granny Smith juice containing 1.14 mM AAP (Table 5). In the absence of APase, AAP was less effective than an equimolar concentration of AA in inhibiting browning (Expt. I). When APase was added, the AAP treatment controlled browning for at least 6 hr (Expt. II).

A further indication that AAP hydrolysis in treated apple juice was insufficient was shown by measurements of residual AAP in Granny Smith juice with 1.14 mM AAP added immediately after juicing (Fig. 3). Initially, the juice contained about 0.3 mM AA, added to prevent browning during sample preparation. During storage, the AAP concentration decreased linearly with time as it underwent hydrolysis to AA. However, AAP hydrolysis was not sufficient to maintain the AA concentration which decreased to trace levels within 2 hr. The

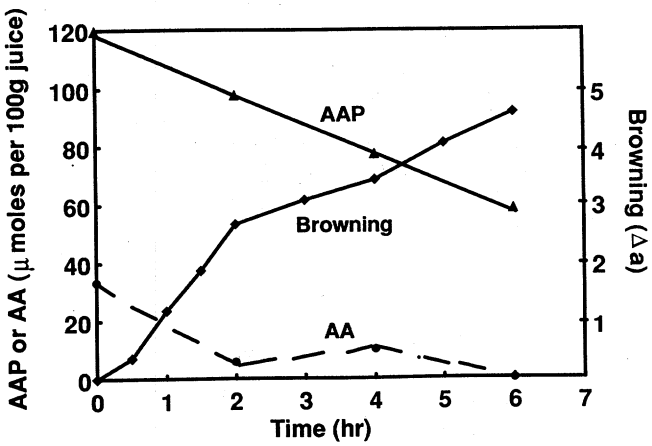

Fig. 3—Concentrations of ascorbic acid-2-phosphate (AAP) and ascorbic acid (AA) and extent of browning (Δa value) in Granny Smith apple juice with 1.14 mM AAP added and stored at 20°C.

onset of browning coincided with the disappearance of AA and became pronounced when more than 50% of the AAP remained unhydrolyzed.

Because of its stability and ability to release AA gradually with relatively little DHAA accumulation, AAP shows considerable potential as a browning inhibitor. Treatments with this compound, however, must be adapted to the special characteristics of each commodity, i.e., their ability to absorb the browning inhibitor, endogenous APase activity, pH and rate of AA oxidation. This might be done by such approaches as use of buffered AAP dips or substitution of ascorbic acid-2-polyphosphates for AAP to obtain the desired hydrolysis rate and use of AAP-AA combinations. Such studies are in progress.

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